Expression of B7 Costimulatory Molecule in Cultured Human Epidermal Langerhans Cells Is Regulated at the mRNA Level

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Langerhans cells (LC) belong to the dendritic cell lineage and are the principal antigen-presenting cells of squamous epithelia. Short-term cultured LC (cLC) exhibit a marked augmented capacity to stimulate allogeneic T cells and acquire the ability to activate naive T cells, probably in relation to enhanced expression of accessory signals. In this study, we evaluated the expression of B7 costimulatory molecule (CD80) in human freshly isolated (fLC) and cLC at both the protein and mRNA level. Staining of frozen skin sections did not reveal any epidermal dendritic cell reactive with either of two different anti-B7 monoclonal antibodies. fLC in suspension did not exhibit any B7 staining as evaluated by two-color flow-cytometry analysis and immunoelectron microscopy. In contrast, LC that were cultured for 24–72 h displayed strong surface B7 reactivity with a characteristic patchy pattern. Treatment with dispase and trypsin did not reduce B7 staining of cLC. Following warming to 37°C, cLC tagged with anti-B7 monoclonal antibody and gold-conjugated secondary antibody could internalize surface B7 by using the organelles of receptor-mediated endocytosis. B7 mRNA, detected by the reverse-transcriptase polymerase chain reaction technique, was expressed at a low level in purified (>90% HLA-DR$^+$) fLC but not in LC-depleted epidermal cells, and was markedly upregulated in purified cLC. The results indicate that 1) fLC do not express B7 protein on their surface, but acquire B7 during culture, 2) surface B7 is not sensitive to trypsin, 3) B7 expression is regulated primarily at the mRNA level, and 4) membrane B7 can be internalized within cLC. B7 molecule on CLC may be relevant to their increased antigen-presenting cell potency and ability to stimulate naive T lymphocytes. *Key words: dendritic cells/antigen presentation/accessory molecules. J Invest Dermatol 103:54–59, 1994*

Epidermal Langerhans cells (LC) belong to the dendritic cell (DC) lineage and are the principal antigen-presenting cells (APC) in squamous epithelia [1,2]. Several *in vitro* and *in vivo* studies have demonstrated that LC can potently present alloantigens, protein antigens, and haptens to T lymphocytes [1–4]. Following isolation from epidermis and short-term culture, LC undergo profound changes in both phenotype and APC functions. Compared to freshly isolated cells (fLC), cultured LC (cLC) express higher amounts of surface major histocompatibility complex (MHC) class I and class II molecules, intercellular adhesion molecule-1 (ICAM-1), and β_2 integrins [1,4]. Functionally, cLC exhibit a marked augmented capacity to stimulate allogeneic T lymphocytes and acquire the ability to activate naive T cells [1,5–6].

In addition to MHC-peptide complexes, APC need to provide accessory signals to activate T cells. The delivery of these costimulatory signals has profound consequences on T-cell–receptor engagement, and the lack of costimulation may lead to functional inactivation of naive T cells [7]. The best characterized costimulatory signal is the B7 molecule (CD80) [8], which binds to CD28 or the CD28 homologous, CTLA-4, on T lymphocytes [9,10]. B7 mRNA is expressed by macrophages and B cells only after long-term activation with interferon-γ or lipopolysaccharide, respectively; in contrast, it seems to be constitutively present in DC [11]. Recently, cultured human peripheral blood DC, as well as human and mouse epidermal LC, have been shown to express functional B7 protein, whereas freshly isolated cells did not [11–14]. In addition, both freshly procured and cultured crude epidermal cell (EC) suspensions contained B7-specific mRNA [14], but it was unclear whether this mRNA was derived only from LC.

In the present study, we investigated B7 molecule expression in human skin sections as well as in fLC and cLC in suspension, and analyzed the internalization of surface B7 in cLC. Furthermore, we evaluated the presence of B7 mRNA in both *purified* fLC and cLC fractions.

MATERIALS AND METHODS

Staining of Skin Sections Frozen 4–6-μm thick sections from both normal adult skin of the buttock and neonatal foreskin were processed for immunohistochemistry using a three-step avidin-biotin-peroxidase technique, as previously described [15]. Primary monoclonal antibodies (MoAb) were anti-CD1a (2.5 μg/ml; OKT6, Ortho Diagnostic System, Raritan, NJ), anti-B7 (10–50 μg/ml, IgG_1; clone L307.4, Becton Dickinson, San Jose, CA) and B7-24 (10–50 μg/ml, IgG_{2a}; kindly provided by Dr. M. de Boer of Innogenetics, Ghent, Belgium) [16]. Control samples were incubated with irrelevant matched isotype MoAb.

EC Suspensions and LC Enrichment Skin was obtained from adult patients undergoing plastic surgery. Keratomized skin pieces were floated in 0.5% dispase (Neutral protease grade II, Boehringer Mannheim, Mannheim, Germany) for 60 min at 37°C. Epidermis was then separated from

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Abbreviations: APC, antigen-presenting cells; RT-PCR, reverse-transcription polymerase chain reaction.

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dermis and single EC suspensions were prepared by trypsinization (0.25% for 20 min at 37°C), as described [17]. EC viability was estimated by trypan blue exclusion to range from 85 to 97%. EC were enriched for LC as described [18]. Briefly, EC suspensions were allowed to adhere to collagen-coated dishes for 90 min; the non-adherent cell fraction was then subjected to cold hypotonic lysis followed by density gradient centrifugation using Ficoll-Paque (1.077 g/ml) (Pharmacia, Uppsala, Sweden). Cells from the interface routinely contained 15–40% LC.

Cell Cultures EC suspensions enriched for LC as described above were cultured for 24–72 h in RPMI 1640 supplemented with heat-inactivated 10% fetal bovine serum (FBS), 25 mM HEPES, 0.1 mM non-essential amino acids, 2 mM L-glutamine, 1 mM sodium pyruvate (all Biochrom KG, Berlin, Germany), 0.05 mM 2-mercaptoethanol (Merck, Daemstadt, Germany), 100 U/ml penicillin, 100 μg/ml streptomycin, and 200 ng/ml human recombinant granulocyte/macrophage colony-stimulating factor (hrGM-CSF) (gift of Dr. G. Corbetta, Sandoz, Italy). The viability of cells after culture ranged from 60 to 70%.

Raji cells (kindly provided by Dr. M. C. Re, Institute of Microbiology, University of Bologna, Italy) represent a Burkitt lymphoma B-cell line that expresses B7 mRNA and protein [19]. Cells were cultured in RPMI 1640 supplemented with 10% FBS, 25 mM HEPES, 2 mM L-glutamine, and antibiotics.

Immunofluorescent Staining of Cell Suspensions Dulbecco's modified Eagle's medium supplemented with 10 mM HEPES and 1% FBS was used to wash cells and dilute antibodies. Freshly isolated or cultured EC enriched for LC were incubated with the MoAb L307.4 (1–10 μg/ml) or B7-24 (10 μg/ml) for 30 min on ice and then with phycoerythrin-conjugated goat anti-mouse Ig (Dakopatts, Copenhagen, Denmark) for 30 min. After incubation with 2% normal mouse serum for 10 min, cells were stained with fluorescein isothiocyanate–conjugated anti–HLA-DR (0.5 μg/10^6 cells) for 30 min on ice, and finally fixed in 3% paraformaldehyde. Raji cells were stained with anti-B7 MoAb (both at 10 μg/ml) for 30 min on ice, followed by phycoerythrin-conjugated goat anti-mouse Ig, and then fixed in 3% paraformaldehyde. Cells were evaluated under a Zeiss Axioscop fluorescent microscope and with a FACScan equipped with Lysys II software (Becton Dickinson), as previously described [20].

Immunoelectron Microscopy Freshly isolated or cultured EC enriched for LC were incubated with anti-B7 (clone L304.7) or an irrelevant isotype-matched MoAb and then with 5-nm gold-conjugated goat anti-mouse IgG (Amersham International, Amersham, UK), as previously described [21]. To study endocytosis of membrane B7, cultured EC were warmed to 37°C for 5–60 min before fixation. Cells were fixed in 1% glutaraldehyde in a Tyrode buffer, post-fixed in 1% osmium tetroxide, dehydrated in graded alcohols, and finally embedded in Durcopan ACM (Fluka, Buchs, Switzerland). Thin sections were stained with uranyl acetate and lead citrate and then examined with a Philips EM 400 electron microscope (Centro Interdipartimentale Grandi Strumenti, Università di Modena). Staining with an irrelevant primary MoAb yielded consistently negative results.

Purification of EC Fractions fLC and cLC were separated from EC suspension by using an indirect immunomagnetic technique, as described in detail elsewhere [22]. Briefly, crude EC suspensions, either freshly isolated or cultured for 48 h, were incubated with anti-CD1a (OKT6) MoAb and then with Dynebeads M-450 coated with sheep anti-mouse IgG_1 (Dynal-SAM) (Dynal, Oslo, Norway). The rosetted cell fraction contained more than 90% HLA-DR^+ cells (i.e., LC) as estimated by flow cytometry [22]. In contrast, no HLA-DR^+ cells could be detected in the non-rosetted EC fraction, corresponding to LC-depleted EC.

Detection of B7 mRNA Total cellular RNA was extracted from 1×10^5 crude freshly procured EC, freshly isolated and 72-h cultured LC-depleted EC, purified fLC and 72-h cLC, and Raji cells using a modification of the guanidium isothiocyanate procedure in the presence of 10 μg of *Escherichia Coli* tRNA [23]. To further purify the RNAs and to avoid genomic DNA contamination, samples were digested with RQ1, 0.05 U/μg RNA, RNAsin (Promega, Madison, WI) 40 U and 10 × RQ1 buffer (0.4 M Tris pH 7.9, 0.1 M NaCl, 6 mM $MgCl_2$, 0.1 mM $CaCl_2$, H_2O to 200 μl). Oligonucleotide primers and probes were synthesized on an automated solid-phase synthesizer (Applied Biosystems, Inc, Mod 381 A), as described [23]. The direct (nt 712–731) and reverse (nt 1177–1196) DNA primers for B7 define a 480-bp cDNA region that spans three introns within the B7 gene [14]. The B7 reverse probe (ATGAGACACATGAAGCTGTGGTTGGTTGTCATATTGAAAT) recognizes region 931–970 [19]. The reverse-transcription polymerase chain reactions (RT-PCR) were carried out as described [23]. Briefly, total cellular RNA was reverse transcribed using 200 U of M-MLV reverse transcriptase (GIBCO BRL, Life Technologies, Gaithersburg, MD) and 1 μg of OligodT 15 primer (Boehringer) for 1 h at 37°C in 1 × cDNA buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2 mM $MgCl_2$, 10 mM dithiothreitol, and 200 μM dNTPs). The resulting specific cDNA fragments were amplified with 2.5 U of Taq polymerase (Promega) in the presence of 0.5 μg primers and 1 × cDNA buffer. DNA fragments corresponding to the different genes studied were generated during 40 cycles of PCR (1 min at 94°C, 2 min at 55°C, and 4 min at 72°C). Fifteen microliters of the PCR reaction mixture (50 μl total volume reaction) were separated on a 2% agarose gel and electroblotted onto a Gene-Screen membrane (NEN, Boston, MA). Blots were hybridized with [^{32}P]-labeled oligodeoxynucleotide probes as described [23]. For each sample studied, the following negative controls were performed: 1) amplification of genomic DNA derived from normal peripheral blood leukocytes (1 μg) or RNA amplification without RT; 2) amplification without template. In all experiments, no amplified fragments due to genomic DNA or cDNA contamination were detected. As a control for the amount of the RNA used as substrate, oligomers specific for the β-actin cDNA [24] were used in parallel RT-PCR reaction. Each experiment was repeated at least three times.

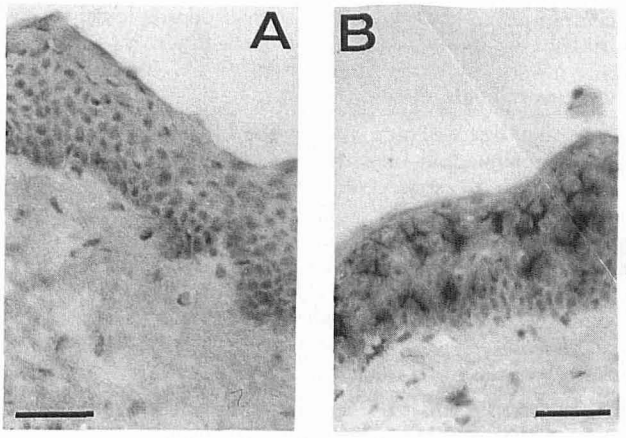


Figure 1. LC *in situ* do not express B7. Frozen sections from normal appearing skin were incubated with either anti-B7 *(A)* or anti-CD1a *(B)* MoAbs and then stained using a three-step avidin-biotin-peroxidase complex amplification system. *Bars,* 20 μm.

RESULTS

cLC, but Neither LC *In Situ* nor fLC, Express B7 Protein Sections of normal human skin stained with either of the two different MoAb against B7 did not reveal any dendritic epidermal positive cell (Fig 1*A*). However, epidermal dendritic cells were clearly visible when stained for CD1a antigen (Fig 1*B*). Two-color flow-cytometry analysis of freshly isolated EC suspensions enriched for LC did not show any B7-positive LC (Fig 2*A*). In contrast, LC cultured for 72 h exhibited a marked expression of B7 (Fig 2*C*), with 75–95% of epidermal HLA-DR^+ cells showing B7 staining. B7 reactivity was already evident on cLC after 24 h of culture and increased progressively during the following 2 d of culture (data not shown). cLC also displayed a fourfold up-regulation of surface HLA-DR molecules compared to fLC (Fig 2, *A* versus *B*), as previously reported [5,25].

Immunoelectron microscopy confirmed the lack of B7 on fLC (data not shown) and its presence on cLC (Fig 3*A,B*). cLC exhibited a certain variability in the number of gold particles present on their surface, with as few as 15 to more than 200 granules per LC. In addition, B7 molecule on cLC surface was characterized by a patchy distribution, with aggregates of 5–30 gold granules mainly localized on the lower part of surface microvilli. Other freshly isolated or cultured EC, including keratinocytes and melanocytes, did not display any B7 reactivity.

Inasmuch as other surface LC molecules, including ICAM-1, lymphocyte function-associated antigen 3 (LFA-3), Pgp-1 (CD44), and E-cadherin are trypsin sensitive [2,26,27], we asked whether the lack of expression of B7 in fLC could be due to its removal by the enzymes used to disaggregate epidermal sheets. However, incubation of cLC or Raji cells with both dispase and trypsin at the same

concentrations and for the same time period employed to obtain fLC did not significantly reduce B7 staining intensity (Table I), thus making it very unlikely that the absence of B7 on fLC is due to enzyme treatment of these cells [11].

cLC Internalize Surface B7 by Receptor-Mediated Endocytosis Immunogold-labeled cLC that were warmed to 37°C for 5–60 min showed capping (Fig 3*C*) to one pole of the cell membrane and internalization of gold granules. The label was progressively visible in coated pits, coated vesicles, and especially in endosomes and vacuoles, as well as in lysosomes (Fig 3*D–F*), the organelles that morphologically characterize receptor-mediated endocytosis. However, no gold particles could be observed within Birbeck granules, suggesting that they are not involved in the internalization process of surface B7.

B7 mRNA Is Present in Purified fLC and Is Upregulated in cLC In the next set of experiments, very highly enriched fractions of fLC and cLC (> 90% HLA-DR$^+$ cells) as well as freshly isolated or cultured EC depleted of LC (no HLA-DR$^+$ cells) [22] were tested for the presence of B7 mRNA by RT-PCR. The results showed that both purified fLC and cLC contain specific B7 mRNA [14]; however, the amount of B7 mRNA in cLC was much higher than in fLC (Fig 4). In contrast, no positive signal could be detected in freshly procured crude EC as well as in freshly isolated and cultured LC-depleted EC.

DISCUSSION

Costimulatory signals play a crucial role in the activity of APC, influencing the outcome of peptide/MHC molecule recognition by T cells [7]. Abrogation of these accessory signals by chemical fixation or ultraviolet radiation may lead to clonal anergy rather than clonal expansion of unprimed or some activated T cells [28–30]. Costimulatory molecules identified on the APC surface include ICAM-1, LFA-3, heat-stable antigen, vascular cell adhesion molecule 1 (VCAM-1), ICAM-2, and B7 [9,31]. B7 belongs to the immunoglobulin superfamily of receptors and can costimulate both CD4$^+$ and CD8$^+$ T lymphocytes [32]. Binding of B7 to CD28 results in increased interleukin 2 production by induction of cytokine transcripts and stabilization of cytokine mRNA [33]. Interestingly, initial activation of T cells enhances B7 expression on APC via MHC class II signaling, and this, in turn, can improve the antigen-presentation capacity of APC [34].

In the present study, we investigated the presence of B7 mRNA and the corresponding protein in human epidermal LC. No epidermal dendritic B7-positive cells could be detected in normal human skin sections, in agreement with data from Simon JC, *et al.*§ In contrast, Vanderberghe *et al* have described epidermal dendritic cells reactive with anti-B7 MoAb [35]. It should be noted, however, that in skin diseases characterized by lymphocytic infiltration (e.g., allergic contact dermatitis) B7-positive epidermal LC are readily detected§ (and our unpublished observations). No B7-positive cells were observed in freshly procured EC suspensions, whereas cLC showed strong B7 reactivity, as previously reported [14]. Furthermore, our results indicate that the failure to detect B7 in fLC is not due to removal of B7 epitopes during the preparation of EC suspensions, as enzyme treatment of either cLC or Raji cells did not reduce B7 staining intensity. As previously suggested with immunofluorescence [14], immunoelectron microscopy confirmed that B7 protein distribution on the cLC membrane is patchy, a pattern that is not shared by other surface proteins such as CD1a or MHC class II molecules. Similar patchy labeling has been described for RFD1 MoAb, which reacts with a D locus product preferentially expressed on human DC [36]. Using RT-PCR, we have shown that *purified* fLC contain low levels of B7 mRNA and that, following culture, LC markedly upregulate B7 mRNA, indicating that B7 expression

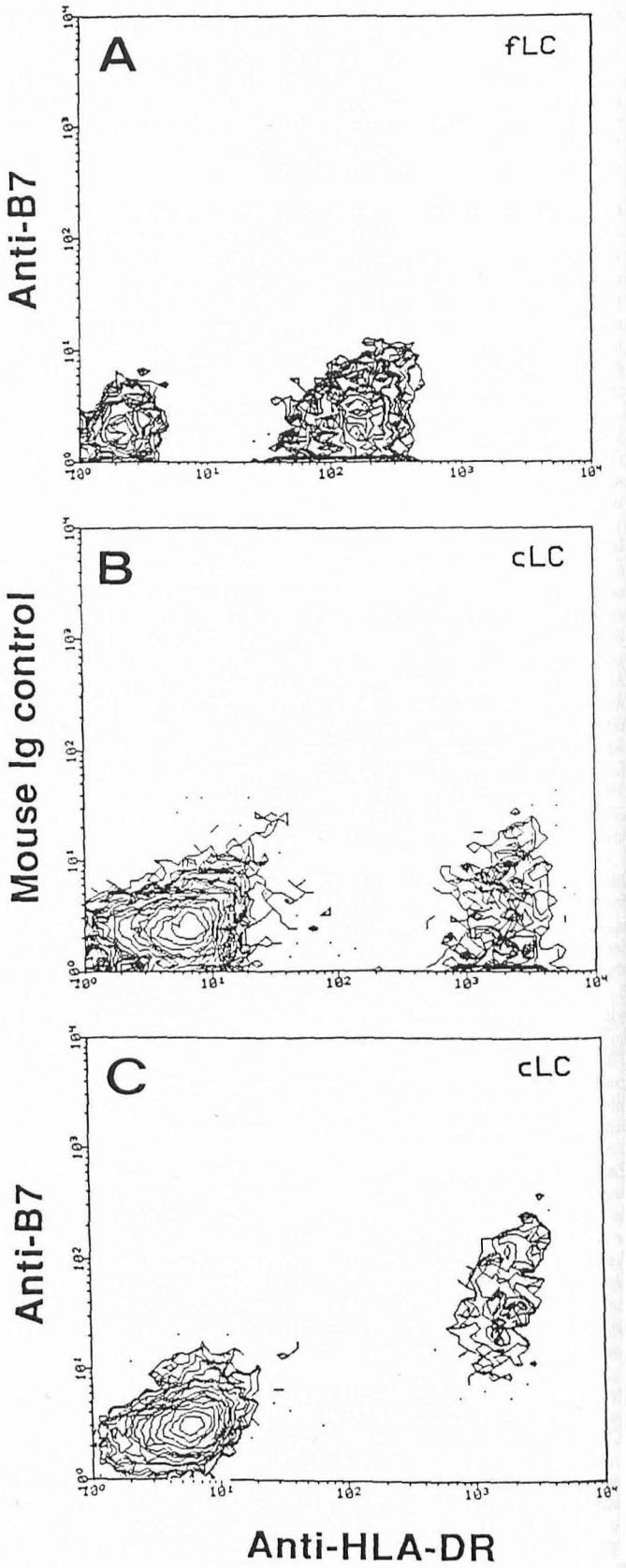
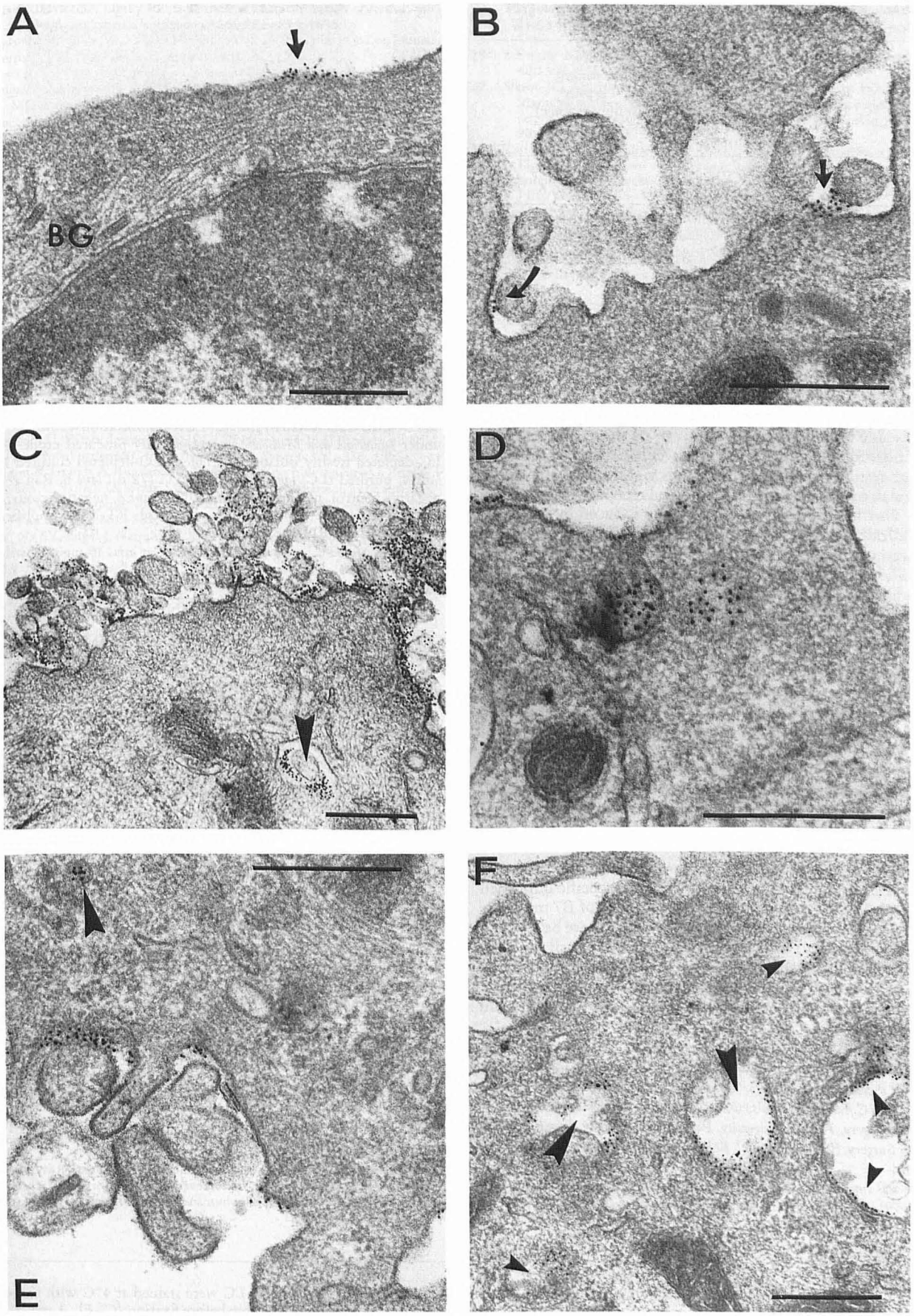


Figure 2. cLC but not fLC express B7. Freshly isolated *(A)* or 72-h cultured epidermal cells enriched for LC were stained with either isotype matched mouse Ig *(B)* or anti-B7 MoAb (L307.4) *(C)* and PE-conjugated goat anti-mouse Ig, followed by fluorescein isothiocyanate–conjugated anti–HLA-DR. Results are representative of three experiments. Fluorescence is expressed in approximately four decades $\log_{10}$ scale.

§ Simon JC, Dietrich A, Mielke V, Augustin M, Vanscheidt W, Ledbetter JA, Linsley PS, Schöpf E, Sterry W: Cutaneous expression of the B7 activation antigen and its ligand CD28: relevance to immune responses generated in skin (abstr). *J Invest Dermatol* 101:442A, 1993.


A
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D
E
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Table I. Treatment with Dispase and Trypsin Does Not Reduce B7 Staining on Cultured LC (cLC) or Raji Cells

	Fluorescence Intensity Before and After Treatment with Dispase and Trypsin[a] (peak channel/mean fluorescence)	
	Before	After
cLC	17.9/90.8	15.9/106.9
Raji cells	41.1/65.5	35.9/56.6

[a] Cultured epidermal cells enriched for LC or Raji cells were treated with 0.5% dispase (60 min, 37°C) followed by 0.25% trypsin (20 min, 37°C). Cells were then stained for B7 (Raji cells) or B7 and HLA-DR (epidermal cells) and analyzed by flow cytometry.

is primarily modulated at the mRNA level. B7 mRNA was exclusively present in LC fractions, whereas it was not detected in LC-depleted EC, thus confirming that LC is the only EC type expressing B7 under normal conditions. In contrast to Symington *et al* [14], but possibly due to the different sensitivity of the PCR technique used, we failed to detect B7 mRNA in freshly procured crude EC. Finally, our results demonstrate that when cLC are incubated at 37°C, membrane B7 undergoes capping and receptor-mediated endocytosis. This finding, although artificially induced by Ab cross-linking, indicates that surface B7 may be subjected to spontaneous internalization, as hypothesized for MHC class II and CD1a molecules [25,37,38]. Endocytosis and recycling to the cell membrane could be additional mechanisms of B7 expression modulation.

B7 upregulation in culture reflects the behavior of other surface molecules involved in antigen presentation, such as MHC molecules, LFA-3, and ICAM-1. There is evidence that GM-CSF and other EC-derived cytokines are at least in part responsible for the phenotypic and functional changes manifested by LC and DC during culture [1,2]. We added GM-CSF to the culture medium because it improves survival of LC in culture; however, B7 upregulation was observed also in the absence of exogenous GM-CSF (not shown). Furthermore, in pathologic conditions,§ lymphocyte-derived cytokines could be responsible for B7 expression by epidermal LC *in vivo*. In this respect, the possibility that also keratinocytes express B7-like molecules under certain "activation" conditions has been suggested [39].

Compared to fLC, cLC exhibit an increased capacity to present non-self MHC antigens and to stimulate hapten-specific unprimed T cells [2,5,6]. We hypothesize that the expression of B7 molecule by cLC is relevant to these functions. As LC and DC are believed to be the most potent APC in the activation of naive T cells and the initiation of T-cell–mediated immune responses, studies on the regulation of accessory molecules present on these APC may greatly help the understanding of how T-cell immunity is generated and regulated.

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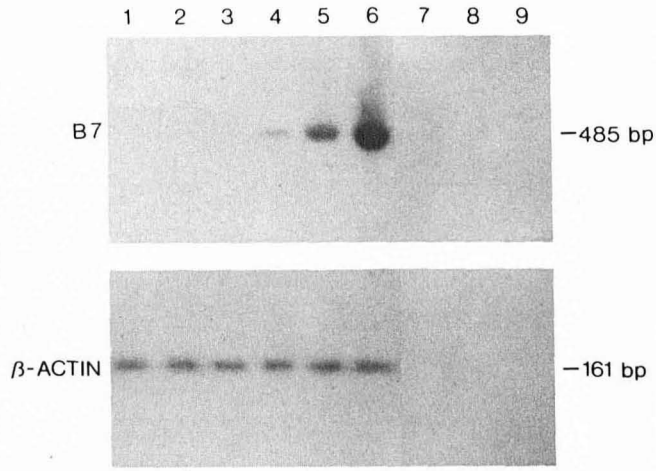

Figure 4. B7 mRNA is present in fLC and is markedly upregulated in cLC. fLC, cLC, and LC-depleted EC were purified by an immunomagnetic technique using anti-CD1a MoAb. Total RNA was extracted from 1×10^5 cells and B7 mRNA detected by the RT-PCR amplification technique, as detailed under *Materials and Methods*. *Lane 1,* freshly procured crude EC; *lane 2,* LC-depleted freshly isolated EC; *lane 3,* LC-depleted cultured EC (72 h); *lane 4,* purified fLC; *lane 5,* purified cLC (72 h); *lane 6,* Raji cells; *lane 7,* negative control, purified genomic DNA; *lane 8,* negative control, purified RNA without RT; *lane 9,* negative control, RT-PCR amplification performed without RNA template.

REFERENCES

1. Romani N, Schuler G: The immunologic properties of epidermal Langerhans cells as a part of the dendritic cell system. *Springer Semin Immunopathol* 13: 265–279, 1992
2. Paglia P, Girolomoni G, Robbiati F, Granucci F, Ricciardi-Castagnoli P: Immortalized dendritic cell line fully competent in antigen presentation initiates primary T cell responses in vivo. *J Exp Med* 178:1893–1901, 1993
3. Cohen PJ, Katz SI: Cultured human Langerhans cells process and present intact protein antigens. *J Invest Dermatol* 99:331–336, 1992
4. Girolomoni G, Pastore S, Zacchi V, Cavani A, Marconi A, Giannetti A: Phosphatidylserine enhances the ability of epidermal Langerhans cells to induce contact hypersensitivity. *J Immunol* 150:4236–4243, 1993
5. Romani N, Lenz A, Glessel H, Stössel H, Stanzl U, Majdic O, Fritsch P, Schuler G: Cultured human epidermal Langerhans cells resemble lymphoid dendritic cells in phenotype and culture. *J Invest Dermatol* 93:600–609, 1989
6. Dai R, Grammer SF, Streilein JW: Fresh and cultured Langerhans cells display differential capacities to activate hapten-specific T cells. *J Immunol* 150:59–66, 1993
7. Liu Y, Linsley PS: Costimulation of T-cell growth. *Curr Op Immunol* 4:265–270, 1992
8. Shaw S, *et al*: Antibodies and molecules of the 5th International Workshop of Leukocyte differentiation antigens. In: Schlossman S, Boumsell L, Gilks W, Harla J, Kishimoto T, Morimoto C, Ritz J, Shaw S, Silverstein R, Springer T, Tedder T, Todd R (eds.). *Leukocyte Typing V: White Cell Differentiation Antigens.* Oxford University Press (in press)
9. Damle NK, Klussman K, Linsley PS, Aruffo A: Differential costimulatory effects of adhesion molecules B7, ICAM-1, LFA-3, and VCAM-1 on resting and antigen-primed $CD4^+$ lymphocytes. *J Immunol* 148:1985–1992, 1992
10. Galvin F, Freeman GJ, Razi-Wolf Z, Hall W, Benaceraff B, Nadler L, Reiser H: Murine B7 antigen provides a sufficient costimulatory signal for antigen-specific and MHC-restricted T cell activation. *J Immunol* 149:3802–3808, 1992
11. Larsen CP, Ritchie SC, Pearson TC, Linsley PS, Lowry RP: Functional expression of the costimulatory molecule, B7/BB1, on murine dendritic cell populations. *J Exp Med* 176:1215–1220, 1992
12. Young JW, Koulova L, Soergel SA, Clark EA, Steinman RM, Dupont B: The B7/BB1 antigen provides one of several costimulatory signals for the activation of $CD4^+$ T lymphocytes by human blood dendritic cells in vitro. *J Clin Invest* 90:229–237, 1992

Figure 3. cLC internalize by receptor-mediated endocytosis membrane B7. 48-h cultured EC enriched for LC were stained at 4°C with anti-B7 MoAb followed by 5-nm gold-conjugated goat anti-mouse IgG and then fixed *(A,B)* or incubated at 37°C for 5–60 min before fixation *(C–F)*. *A* and *B*, clusters of gold granules on the plasma membrane *(arrows)*; BG, Birbeck granules. *C*, capping of B7 to one cell pole following incubation at 37°C for 10 min; note the presence of gold granules within a cytoplasmic vacuole *(arrowhead)*. *D–F*, cLC showing gold particles on the membrane as well as inside endosomes and vacuoles *(arrowheads)*. *Bars*, 0.5 μm.

13. Thomas R, Davies LS, Lipsky PE: Isolation and characterization of human peripheral blood dendritic cells. *J Immunol* 150:821–834, 1993
14. Symington FW, Brady W, Linsley PS: Expression and function of B7 on human epidermal Langerhans cells. *J Immunol* 150:1286–1295, 1993
15. Giannetti A, Girolomoni G, Pincelli C, Benassi L: Immunopathologic studies in pityriasis lichenoides. *Arch Dermatol Res* 280:S61–S65, 1988
16. de Boer M, Parren P, Dove J, Ossendrop F, van der Horst G, Reeder J: Functional characterization of a novel anti-B7 monoclonal antibody. *Eur J Immunol* 22:3071–3075, 1992
17. Girolomoni G, Santantonio ML, Pastore S, Bergstresser PR, Giannetti A, Cruz PD: Epidermal Langerhans cells are resistant to the permeabilizing effects of extracellular ATP: in vitro evidence supporting a protective role of membrane ATPase. *J Invest Dermatol* 100:282–287, 1993
18. Wang B, Rieger A, Kilgus O, Ochiai K, Maurer D, Födinger D, Kinet J-P, Stingl G: Epidermal Langerhans cells from normal human skin bind monomeric IgE via FcεRI. *J Exp Med* 175:1353–1365, 1992
19. Freeman GJ, Freedman AS, Segil JM, Lee G, Whitman JF, Nadler LM: B7, a new member of the Ig superfamily with unique expression on activated and neoplastic B cells. *J Immunol* 143:2714–2722, 1989
20. Cossarizza A, Ortolani C, Forti E, Montagnani G, Paganelli R, Zannotti M, Marini M, Monti D, Franceschi C: Age-related expansion of functionally inefficient cells with markers of natural killer activity in Down's syndrome. *Blood* 77:1263–1270, 1991
21. Girolomoni G, Zambruno G, Manca V, Bergstresser PR, Gianetti A, Cruz PD: Spleen dendritic cells exhibit altered morphology and increased allostimulatory capacity after short term culture. *Exp Dermatol* 1:129–133, 1992
22. Giannetti A, Zambruno G, Cimarelli A, Marconi A, Negroni M, Girolomoni G, Bertazzoni U: Direct detection of HIV-1 RNA in epidermal Langerhans cells of HIV-infected patients. *J Acquir Immune Defic Syndr* 6:329–333, 1993
23. Manfredini R, Grande A, Tagliafico E, Barbieri D, Zucchini P, Citro G, Zupi G, Franceschi C, Torelli U, Ferrari S: Inhibition of c-fes expression by an antisense oligomer causes apoptosis of HL60 cells induced to granulocytic differentiation. *J Exp Med* 178:381–389, 1993
24. Ponte P, Ng SY, Engel J, Gunning P, Kedes L: Evolutionary conservation in the translated regions of actin mRNAs: DNA seuqence of a human beta-actin cDNA. *Nucleic Acid Res* 12:1687–1696, 1984
25. Girolomoni G, Cruz PD, Bergstresser PR: Internalization and acidification of surface HLA-DR molecules by epidermal Langerhans cells: a paradigm for antigen processing. *J Invest Dermatol* 94:753–760, 1990
26. Aiba S, Nakagawa S, Ozaka H, Miyake K, Yagita H, Tagami H: Up-regulation of α4 integrin on activated Langerhans cells: analysis of adhesion molecules on Langerhans cells relating to their migration from skin to draining lymph nodes. *J Invest Dermatol* 100:143–147, 1993
27. Tang A, Amagai M, Granger LG, Stanley JR, Udey MC: Adhesion of epidermal Langerhans cells to keratinocytes mediated by E-cadherin. *Nature* 361:82–85, 1993
28. Schwartz RH: A cell culture model for T lymphocyte clonal anergy. *Science* 48:1349–1353, 1990
29. Simon JC, Krutmann J, Elmets CA, Bergstresser PR, Cruz PD: Ultraviolet B–irradiated antigen presenting cells display altered accessory signaling for T-cell activation: relevance to the immune responses initiated in skin. *J Invest Dermatol* 98:66S–69S, 1992
30. Tan P, Anasetti C, Hansen JA, Melrose J, Brunvand M, Bradshaw J, Ledbetter JA, Linsley PS: Induction of alloantigen-specific hyporesponsiveness in human T lymphocytes by blocking interaction of CD28 with its natural ligand B7/BB1. *J Exp Med* 177:165–173, 1993
31. Liu Y, Jones B, Aruffo A, Sullivan KM, Linsley PS, Janeway CA: Heat-stable antigen is a costimulatory molecule for CD4 T cell growth. *J Exp Med* 175:437–445, 1992
32. Townsend SE, Allison JP: Tumor rejection after direct costimulation of $CD8^+$ T cells by B7-transfected melanoma cells. *Science* 259:368–370, 1993
33. Norton SD, Zuckerman L, Urdhal KB, Shefner R, Miller J, Jenkins MK: The CD28 ligand, B7, enhances IL-2 production by providing a costimulatory signal to T cells. *J Immunol* 149:1556–1561, 1992
34. Watts TH, Alaverdi N, Wade WF, Linsley PS: Induction of costimulatory molecule B7 in M12 B lymphomas by cAMP or MHC-restricted T cell interaction. *J Immunuol* 150:2192–2202, 1993
35. Vanderberghe P, Delabie J, de Boer M, De Wolf-Peeters C, Ceuppens JL: In situ expression of B7/BB1 on antigen-presenting cells and activated B cells: an immunohistochemical study. *Int Immunol* 5:317–321, 1993
36. Knight SC, Freyer P, Griffiths S, Harding B: Class II histocompatibility antigens on human dendritic cells. *Immunology* 61:21–27, 1987
37. Hanau D, Fabre M, Schmitt DA, Garaud J-C, Pauly G, Tongio M-M, Mayer S, Cazenave J-P: Human epidermal Langerhans cells cointernalize by receptor-mediated endocytosis "nonclassical" major histocompatibility complex class I molecules (T6 antigens) and class II molecules (HLA-DR antigens). *Proc Natl Acad Sci USA* 84:2901–2905, 1987
38. Bucana CD, Munn CG, Song MJ, Dunner K, Kripke ML: Internalization of Ia molecules into Birbeck granule-like structures in murine dendritic cells. *J Invest Dermatol* 99:365–373, 1992
39. Augustin M, Dietrich A, Niedner R, Kapp A, Schöpf E, Ledbetter JA, Brady W, Linsley PS, Simon JC: Phorbol-12-myristate-13-acetate-treated human keratinocytes express B7-like molecules that serve a costimulatory role in T-cell activation. *J Invest Dermatol* 100:275–281, 1993